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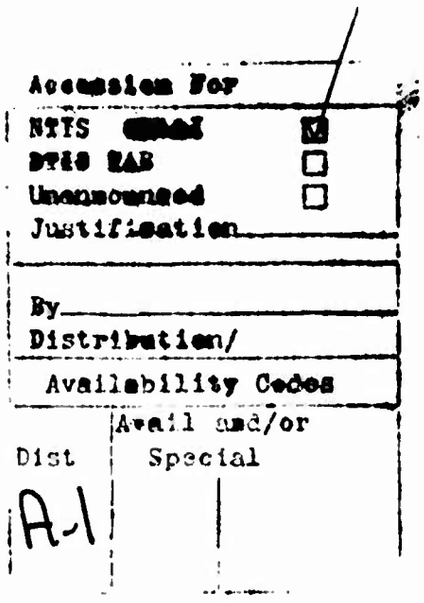

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Circular dichroism studies of tryptophan residues in gramicidin

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Introduction

Gramicidin is a linear polypeptide produced by *Bacillus brevis* which forms cation-selective channels in lipid membranes. The sequence of the main component (Val^1 -Gramicidin A) is: HCO-L-Val^1-Gly^2-L-Ala^3-D-Leu^4-L-Ala^5-D-Val^6-L-Val^7-D-Val^8-L-Trp^9-D-Leu^{10}-L-Trp^{11}-D-Leu^{12}-L-Trp^{13}-D-Leu^{14}-L-Trp^{15}-NHCH$_2$CH$_2$OH.

It is generally accepted that the conducting channel consists of two gramicidin monomers linked by hydrogen bonds between their amino-termini [1]. The polypeptide backbone of each monomer is believed to adopt a $\beta^{6.3}$ helical conformation; this provides a pathway for ion movement through the center of the helix where peptide carbonyl groups provide ion coordination sites. All side chains are on the outside of the helix where they can interact with the lipid membrane. Although not in direct contact with ions, the side chains nevertheless play a significant role in the transport process. For instance, substitution of Trp^{11} by Phe or Tyr (gramicidin B and gramicidin C respectively) results in channels with different lifetimes and conductances although the $\beta^{6.3}$ helical conformation remains intact [2]. Modification of Trp residues by N-formylation of the indole rings results in inactivation of gramicidin channels although again the $\beta^{6.3}$ helix appears intact [3]. The role of Trp side chains in ion transport must presumably result from effects of side chain conformational states and mobility on the conformational dynamics of the backbone and/or effects of the indole ring dipoles on the electrical characteristics of the channel. Furthermore, since they are in direct contact with lipid, Trp side chains can respond to bilayer properties (tension, thickness, fluidity) which modulate gramicidin channel function.

Besides acting as an ion channel, gramicidin has other membrane-modifying properties including effects on lipid order, phase properties and membrane fusion. Trp residues have been shown to be essential for all of these effects [4]. It is clear then that information pertaining to the conformational state of these residues is important in understanding function. We have studied the circular dichroism (CD) observed in the near-UV region (340–240 nm) which is sensitive particularly to the three-dimensional arrangement of the (four) Trp side-chains in the molecule without contributions from the peptide bond chromophores.

Results and Discussion

The near-UV CD spectrum of gramicidin D (80% gramicidin A) in ethanol is shown in Fig. 1. There is a broad, rather weak positive ellipticity between 260 and 300 nm with maxima at about 290 and 297 nm. This is comparable to what has previously been reported for gramicidin in organic solution [5]. The Trp ^1L_b bands are responsible for the fine structure in the longer wavelength region and the broad, featureless peak is attributed to ^1L_a bands. The peptide concentration employed has been shown to result in a mixture of four dimeric forms of gramicidin in this solvent with an anti-parallel double helix being predominant [6]. In the crystal structure of this form, Trp residues are generally not in close proximity and tend to occur in orthogonal pairs [7]; this may be responsible for the rather weak ellipticity. The near-UV CD spectrum of gramicidin in TFE or in a mixture of acetone and DMSO (not shown) where the peptide has been shown to be monomeric and without periodic structure [8] is featureless with an ellipticity near zero.

In contrast, the near-UV CD spectrum of gramicidin in dimyristoyl-phosphatidylcholine (DMPC) vesicles (Fig. 1), in which the peptide is in the channel form [1], has a pronounced negative ^1L_a ellipticity with minima at 285 and 292 nm and local maxima at about 290 and 297 nm corresponding to the ^1L_b transitions observed in ethanol. We have also observed this pattern with dipalmitoyl- and dipalmityl-(ether-linked) phosphatidylcholines (not shown). This clearly indicates a different arrangement of Trp residues than that occurring in solution. Indeed none of the four dimeric forms which occur in organic solvents has a negative ^1L_a ellipticity [5]. Interestingly, the CD pattern we observe in lipids is similar to that found for the model dipeptide H-Trp-Trp-OH in basic methanol [9]. In that case, besides negative ellipticity in the near UV region, a Trp exciton interaction was observed centered on the indole B_b transition at about 225 nm. The far-UV CD of gramicidin in lipids also shows evidence of an exciton interaction centered on this band [10] although peptide chrom-

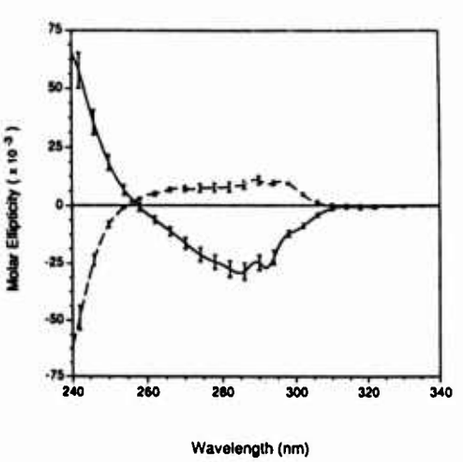

Fig. 1. (Dashed line) Near-UV CD spectrum of gramicidin (7.6 mg/ml) in ethanol at room temperature. Error bars indicate the standard deviation between three separate preparations (10 scans averaged per experiment). (Solid line) Spectrum of gramicidin (0.08 mg/ml) in dimyristoyl-phosphatidylcholine (DMPC) vesicles (0.83 mg/ml lipid) at 30°C. Standard deviation is for five separate preparations.

ophores also play a role in this region. Model-building studies of the gramicidin channel have suggested a close apposition of Trp^9 and Trp^{15} side chains in a 'stacking' interaction. Because of their proximity, these residues would be likely candidates for the putative exciton coupling. If this is the case, the relative orientation of these chromophores must not be strictly parallel (or perpendicular) as these conformations would have a zero exciton signal.

One might expect exciton effects to occur with the L transitions as well; however, both in the dipeptide case and here with the channel form of gramicidin, these couplings are not clearly evident in this region of the spectrum. While there is a crossover point at about 258 nm, there is no obvious transition centered on this wavelength; the positive ellipticity in the region of 235 nm has been attributed to an indole ^1C transition [11]. In the case of the Trp-Trp dipeptide the negative ellipticity in the near-UV region was attributed to a restriction of the side-chain torsional angles χ_1 and χ_2 although one might expect their actual values and not just their variability to be important. Raman spectroscopy has indicated a narrow range of Trp χ_2 angles in the channel form of gramicidin [12]. This corroborates energy calculations which have indicated large barriers for side chain rotation [13]. Indeed the near-UV CD pattern we observe persists at least to 70°C which is a further indication of rather rigid side chain conformation(s). This rigidity indicates that detailed information on side chain conformation which may be obtained from X-ray studies of gramicidin/lipid co-crystals [14] could be directly relevant to the role these sidechain conformers play in gramicidin function under physiological conditions.

Acknowledgements

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